A novel approach to entrapment in alginate – silicate sol – gel matrix

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Introduction

The immobilization of biological material by entrapment in calcium alginate gel is a well-known technique. Alginates are one of the most frequently used polysaccharides due to their low cost, biocompability, and extraordinary easy for immobilization. Enzymes are usually entrapped by drop-wise addition of an aqueous solution of alginate and the biocatalyst to a calcium chloride solution as a hardening bath.

However, application this method to enzyme immobilization suffers from the drawback due to possibility of enzyme leakage from the gel. High levels of enzyme leakage from calcium alginate matrix were often reported (Bodalo 1991, Ro 1991, Boadi 2001, Mittal 2005). To overcome this problem, various methods have been attempted. The enzymes are chemically modified by attaching polymer chain, which makes the enzyme molecule large enough to be retained in the matrix. Other attempts comprise copolymerization and coating the surface of the gel beads (Fadnavis 2003).

Among the strategies used to improve alginate beads properties the strengthening with silica or the silicification of biopolymer was reported by several authors (Coradin 2003). The first attempt to associate silica and alginate for immobilization of enzymes was reported by (Fukushima 1988). The use of tetramethoxysilane (TMOS) as the silica source for immobilization of β–galactosidase in alginate was first reported by (Heichal – Segal 1995). Wet calcium alginate beads were suspended in a solution of TMOS in hexane. Aggregates of silica particles were found throughout the bead. Mature alginate – silicate beads contained 30–40 % w/w silicate.

The same method was applied by (Hsu 1997), (Chen 1998), and (Kim 2001) for immobilization of lipoxygenase, biliburin oxidase, and phospholipase A_2 respectively. Enzymatic activities were comparable to their free state but their thermal and chemical stabilities were improved. Silica gel was also used (Konsoula 2006, Xu 2006) for immobilization of enzymes in alginate gel matrix.

Coating the surface of the alginate gel beads by silica was reported by (Sakai 2001, 2002). They used a mixture of TMOS and 3 – aminopropyl – trimethoxysilane to obtain a silica membrane on calcium alginate beads. The methoxy groups of the TMOS were hydrolysed to form a silica gel and the positively charged amino groups interacted with the alginate surface to deposit mineral. The mineral layer improves the mechanical resistance in comparison to alginate or alginate/poly – lysine beads.

Little information could be found on improvement of entrapment efficiency by using silica for modification of alginate gel. In this report we studied the influence of silicification of alginate gel on the entrapment efficiency of polyvinylpyrrolidone as a model macromolecule.

Materials and methods

Materials

Low viscosity sodium alginate from *Macrocystis pyrifera* and tetramethoxysilane $Si(OCH_3)_4$ (TMOS) were purchased in Sigma – Aldrich. Polyvinylpyrrolidones PVP 40 (MW 40 kDa), PVP 360 (MW 360 kDa) and remazol brilliant blue R (MW 627 Da) were also provided by Sigma–Aldrich. All other chemicals used were of reagent grade.

Methods

Polyvinylpyrrolidones were coloured by the dye remazol brilliant blue R following a procedure described in the literature (Preston 1980). Labelled PVP was prepared by heating PVP (5g)

and remazol brilliant blue (0.375g) in NaOH (100ml, 0.05M) at 50°C for 3 days. The solution of labelled polymer was neutralized by 1M HCl and used without purification.
Sodium alginate solutions 2.0% (w/w) with labelled polyvinylpyrrolidones as markers were prepared in deionised water. The weight of alginate powder was corrected for inherent moisture content. Four experimental procedures were used (Fig. 1) for entrapment of labelled polyvinylpyrrolidones.

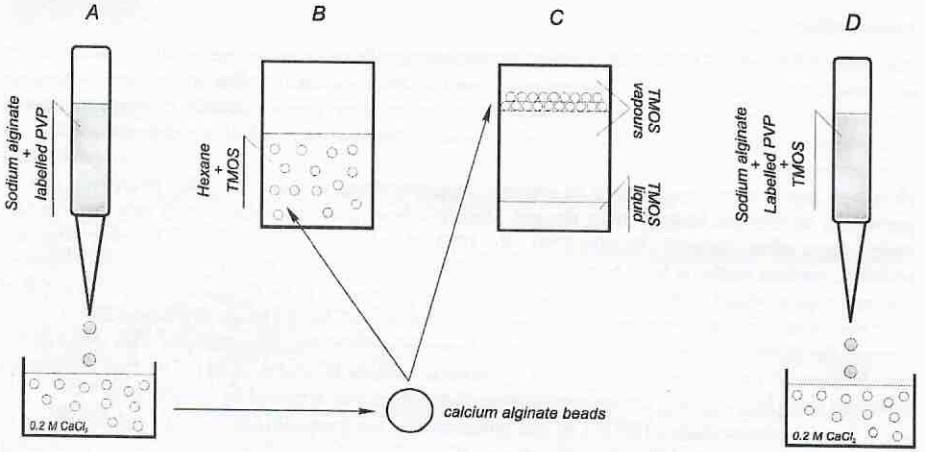

Fig. 1. Procedures used in entrapment of labelled PVP: A – Alginate gel, B – Alginate gel modified by TMOS in hexane, C – Alginate gel modified by vapours of TMOS, D – Copolymerization of alginate and silica.

Procedure A: entrapment in alginate gel. Alginate solutions were extruded through a needle with external diameter 1.2 mm. Ten drops of this mixture were introduced into 5 ml of 0.2 M calcium chloride solution. The hardening solution was maintained under constant stirring using a magnetic stirrer. The release of labelled polyvinylpyrrolidones from beads into calcium chloride solution was determined by measuring spectrophotometrically absorbance in the visible light spectrum region after 24 hours. The content of PVP in alginate solution was also determined spectrophotometrically.
Procedures B and C: entrapment in alginate gel modified by silica. The beads formed (Procedure A) were cured in the gelation media for 3 min and then taken out.
Procedure B. Ten beads were transferred to a glass vessels containing 1 ml hexane. TMOS (1 vol/vol of the beads) was added and the vessels was left for 24h at room temperature.
Procedure C. The beads were cured by vapours of TMOS for 24h at room temperature.
Procedure D: entrapment by copolymerization of alginate and silica. Equal volumes of TMOS and water were vigorously mixed for 1 min followed by mixing with alginate solution. This mixture was deaerated and added drop-wise into 0.2 M $CaCl_2$ solution. The beads were left in the $CaCl_2$ solution for 3 min and then taken out.
The PVP loss was quantified by measuring the absorbance of the gelation and leakage media solution at 592nm using a UV–visible spectrophotometer (JASCO, V–530). The leakage of PVP was determined for ten beads using 5ml of 0.2 M $CaCl_2$ solution.

Results and discussion

The entrapment efficiency of polyvinylpyrrolidones in an alginate – silicate sol – gel matrix was studied. The silica precursor chosen in this study was tetramethoxysilane. The gelation of silica occurs through hydrolysis and polycondensation of precursor. In the sol – gel process the hydrolysis of alkoxides is catalysed by acids, bases or fluoride salts. This study shows that the same effect could be obtained by mixing TMOS with an aquaeous alginate solution. Deposit of mineral in the alginate matrix can be obtained, when alginate beads were cured both by vapours of TMOS and solution of silane in hexane.

Entrapment efficiency of PVP was calculated from equation

$$EE = \left(1 - \frac{m_1 + m_2}{m_0}\right) \cdot 100\%$$

where

m_0 – initial mass of marker in the beads,

m_1 – mass of marker released in gelation media,

m_2 – mass of marker released in leakage media.

The purpose of the leakage assay was to evaluate the leaching of labelled PVP from the beads to the calcium chloride solution over a period of time to consider their potential use in a long – term operation. Several researchers have reported the loss of enzymes in the alginate gel formation process, which varied from approximately 30 to 90% when different enzymes have been entrapped (Bodalo 1991, Ro 1991, Konsoula 2006, Xu 2006). The results for procedure A (Figs 2 and 3) show that the entrapment efficiency in alginate gel matrix depends significantly on molecular weight of PVP and is com- parable with the entrapment efficiencies of enzymes. Modifications of alginates with silica improves entrapment efficiency in all cases (Figs 1 and 2).

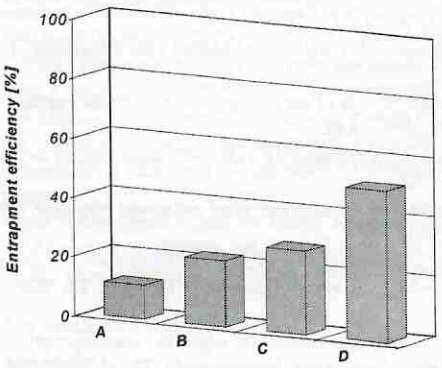

Fig. 2. Entrapment efficiency of PVP 40 for used procedures A, B, C, and D.

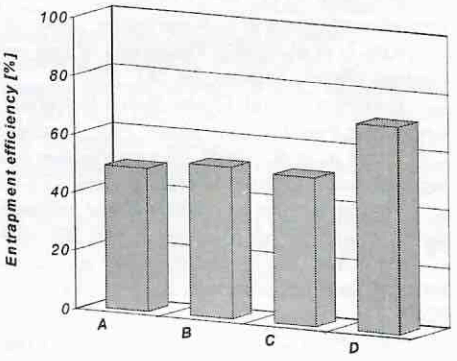

Fig. 3. Entrapment efficiency of PVP 360 for used procedures A, B, C, and D.

This is more pronounced for PVP 40 than for PVP 360. The highest entrapment efficiency could be obtained by using copolymerization of alginate and silica. It was suggested (Xu 2006) that the hydrolysis and condensation of TMOS should be accelerated by alginate molecule.

The catalytic effect of alginate on the biocomposite formation may be due to acceleration of the precursor hydrolysis through carboxyl and nucleation of the silica shell around it. In the biocomposite the PVP molecule may be entrapped in the moiety of the alginate gel, in the moiety of the silica particle, or at the interface of alginate silica (Xu 2006).

Conclusions

Modification of alginate by silica appears very promising approach to improve properties of capsules. The obtained biocomposites showed higher entrapment efficiency of macromolecules than alginate gel. It can thus be believed that alginate – silica biocomposites will find promising applications in biotechnology.

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Acknowledgement

This work was partially supported by grant 3 T0 9C 016 27 from Polish State Committee for Scientific Research.